Multivariate detection of hybridization using conifer terpenes II: Analysis of terpene inheritance patterns in *Pseudotsuga menziesii* F_1 hybrids

Robert P. Adams

Biology Department, Baylor University, Box 97388, Waco, TX 76798, USA,
Robert_Adams@baylor.edu

and

Michael U. Stoehr

British Columbia Ministry of Forest, Lands and Natural Resource Operations,
P. O. Box 9518 Stn Prov. Govt., Victoria, BC, V8W 9C2, Canada

ABSTRACT

The compositions of the volatile leaf oils of *Pseudotsuga menziesii* var. *menziesii* and var. *glauca* and their hybrids were examined and utilized to examine multi-variate methods for the detection of hybridization. The first cross (coastal parent 226 x inland parent 267) produced four hybrids whose oils were much like the inland (var. *glauca*) parent, and 6 hybrids with composition more intermediate between the parents. Eleven of the terpenes were generally intermediate in the hybrids, whereas the other 15 terpenes were transgressive. Truncation of the terpene values to those of the maximum or minimum value of the parents improved PCO ordination using character weights of Fs from ANOVA. A second set of hybrids between coastal parent 517 x intermediate parent 521 yielded similar kinds of variation in the terpenes of the hybrids. Only 2 compounds were intermediate between parents whereas 8 terpenoids showed dominance with values like one of the parents. Nine of the terpenes and percent oil yield were transgressive to both parents. Using F weighting and truncation of transgressives, improved the ordination separating parents from hybrids. However, again, the hybrids were ordinated in two groups. Selecting a balanced set of discriminating terpenes, aided in ordination. In a study of natural field hybridization, the terpenes should be useful to classify the hybrids, but it may be difficult to accurately classify back-crossed or F_2 individuals. Published on-line: **www.phytologia.org** *Phytologia 95(1): 42-57 (Feb. 1, 2013).*

KEY WORDS: *Pseudotsuga menziesii*, *P. m.* var. *glauca*, Douglas-fir, coastal, inland, hybrids, essential oil, terpenes, inheritance, genetics, multi-variate methods.

There are few studies on methods for the detection of hybridization using conifer terpenes from known crosses. The literature was recently reviewed (Adams and Tsumura, 2012). However, in general, Adams (1982) used leaf terpenoids to compare Wells' hybrid distance diagrams, PCA, PCO, and canonical variate analysis, but he had to use putative natural hybrids in *Juniperus*. He found that PCO, using character weighting of F-1 (F ratios from ANOVA between the putative parents), was the most effective method tested.

Confounding the problem is the fact that terpenes are inherited both as intermediate values (additive multi-gene quantitative) and as simple dominant recessive characters as well as transgressive values for many terpenes in a complex mixture (Adams and Tsumura, 2012).

Adams and Tsumura (2012) reported on the leaf volatile oils of two cultivars of *Cryptomeria japonica*, cv. Haava and cv. Kumotooshi, along with their 22 hybrids. The oil of Haava contains appreciable amounts of cis-thujopsene, widdrol and cedrol (not found in Kumotooshi oil) that appear to

be inherited as a linked group in the hybrids in a Mendelian fashion, with a second (dominant/ recessive) gene involved. PCO (Principal Coordinates analysis) using character weights of Fs (F-ratios from one-way ANOVA between the parents) was found to be the most effective method to separate the parents and their hybrids. PCA (Principal Components Analysis) and PCO using equally weighted characters were found to be ineffective in detecting hybrids, as too much weight is given to characters that do not vary among parents. In *Cryptomeria*, Adams and Tsumura (2012) found that the hybrids clustered in two groups: those with and those without the cis-thujopsene/ widdrol/ cedrol suite and that several hybrids' oils were very similar to the Haava parents' oil (Fig. 1).

Both quantitative variation and simple dominance have been reported in the inheritance of terpenes of Douglas fir (von Rudloff, 1984; von Rudloff and Rehfeldt, 1980) and Scots Pine (Pohjola, et al., 2006).
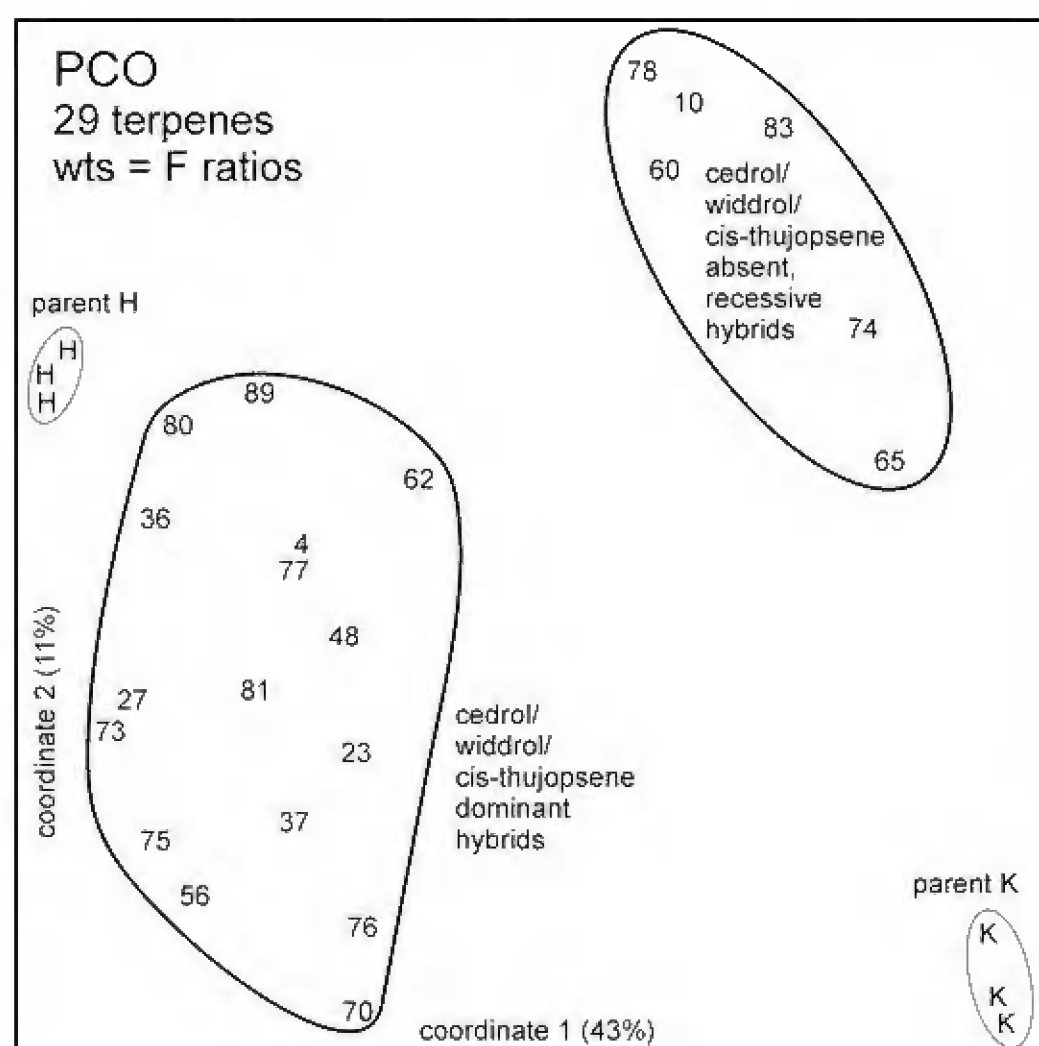

Figure 1. PCO ordination of *Cryptomeria japonica* cv. Haava . and cv. Kumotooshi, along with their 22 hybrids. Note the hybrids fall in two groups.(taken from Adams and Tsumura 2012).

The terpene patterns of Douglas-fir showing inland and coastal groups have recently been re-confirmed (Adams et al. 2012) as the study was extended to cover the entire range of Douglas-fir to southern Mexico. Only two major chemical types were found (inland or interior and coastal).

The purposes of the present paper are to report on a complete analysis of the volatile leaf essential oil of two cultivars of *Pseudotsuga menziesii* var. *menziesii* (coastal) and var. *glauca* (inland) and their F_1 hybrids, and to compare various multivariate methods in the recognition of hybrids using terpenoid data.

MATERIALS AND METHODS

Numerous crosses have been made between Doug Fir accessions as part of the tree improvement program by the British Columbia Ministry of Forests, Lands and Natural Resource Operations (FLNRO). Some of these crosses (and hybrids) involved coastal and inland Doug Fir. Two crosses (#16 and 42) were especially suited for the study of the inheritance of terpenes. Leaves (2 branchlets, 15-20 cm long) were collected from the four parental genotypes growing as grafts in clone banks at the Cowichan Lake Research Station, Mesachie Lake, BC (48° 49'N, 124° 08'W, 160 m), while the hybrids were collected in a single-tree randomized complete block-designed progeny test at Ladysmith, BC (49° 0', 123° 31', 150 m) when the trees were 17 y old. Cross 16: coastal parent 226 (50° 03', 125° 0', 100 m) (*Adams 13111, 13200*) x inland parent 267 (46° 35'N, 123° 05'W, 1200 m) *Adams 13116, 13201*), and F_1 hybrids - *Adams 13204* (map rec. 1133), *13205* (map rec. 827), 13206 (map rec. 694), 13207 (map rec. 669), 13208 (map rec. 587), 13209 (map rec. 565), 13210 (map rec. 639), 13211 (map rec. 1047), 13212 (map rec. 285), 13213 (map rec. 314). Cross 42: coastal parent 517 (52° 49'N, 126° 58'W, 100 m) (*Adams 1312413202*) x intermediate parent 521 (52° 36'N, 127° 10'W, 200 m) (*Adams 13125, 13203*), *Adams*

13214 (map rec. 1208), *Adams 13215* (map rec. 828), *Adams 13216* (map rec. 490), *Adams 13217* (map rec. 623), *Adams 13218* (map rec. 558), *Adams 13219* (map rec. 1182), *Adams 13220* (map rec. 1113), *Adams 13221* (map rec. 1192), *Adams 13222* (map rec. 890), *Adams 13223* (map rec. 287). Voucher specimens are deposited in the Herbarium, Baylor University (BAYLU).

Air dried (30ºC, 24h) leaves (200 g) were steam distilled for 2 h using a circulatory Clevenger-type apparatus and trapped in a layer of diethyl ether (Adams, 1991). The oil samples were concentrated with nitrogen and the samples stored at -20ºC until analyzed. The extracted leaves were oven dried (100ºC, 48 h) for determination of oil yields.

The oils were analyzed on a HP5971 MSD mass spectrometer, scan time 1/ sec., directly coupled to a HP 5890 gas chromatograph, using a J & W DB-5, 0.26 mm x 30 m, 0.25 micron coating thickness, fused silica capillary column (see Adams, 2007 for operating details). Identifications were made by library searches of our volatile oil library (Adams, 2007), using the HP Chemstation library search routines, coupled with retention time data of authentic reference compounds. Quantitation was by FID on an HP 5890 gas chromatograph using the HP Chemstation software with a J & W DB-5, 0.26 mm x 30 m, 0.25 micron coating thickness, fused silica capillary column run under the same conditions as the GCMS analysis (above).

Terpenoids (as percentage of total oil) were compared between the parents (3 replicate per parental genotype) by one-way ANOVA separately for each of the two crosses and SNK (Student-Newman-Keuls) analyses as described by Steele and Torrie (1960). Gower or Manhattan metric similarities (Gower, 1971; Adams, 1975) were computed among all individuals using character weighting of F (where F=MS among parents/MS within parents. MS were taken from ANOVA), and equal weights (wts = 1.0). Using F for weighting factors places more importance on characters that vary more among the two parents, thus improving their separation. Principle Component Analysis (PCA) and Principal Coordinate Ordination (PCO) were performed by factoring the associational matrix using the formulation of Gower (1966) and Veldman (1967).

RESULTS AND DISCUSSION

As reported by von Rudloff (1984) and von Rudloff and Rehfeldt (1980), coastal (var. *menziesii*) and inland (var. *glauca*) Douglas-fir differ in their concentrations of santene, camphene, β-pinene, δ-3-carene, α-terpineol, bornyl acetate, citronellyl acetate, geranyl acetate, germacrene D, α-cadinol and manool (Table 1) as well as many of the smaller components. Hybrids of cross #16, 1, 3 and 8 were generally intermediate in their composition between the parents (Table 1), whereas hybrids 2 and 9 were more like the inland parent (I267, Table 1).

Examination of variation among the hybrids reveals that only 11 terpenes were inherited as intermediate characters (Fig. 2). Several patterns are apparent (Fig. 2). In santene, tricyclene, camphene, β-pinene, 3-carene, borneol and bornyl acetate, the hybrids are generally quite intermediate between the parents (Fig. 2). However, for manool, the tendency is for the hybrids to be very much like one parent (coastal 267 in these cases), in which the compound is missing or present in trace amounts. This is suggestive of Mendelian inheritance, with absence being dominant for manool.

The balance of the 15 compounds (including percent oil yield) show transgressive variation in the hybrids (Fig. 3). Sabinene is an extreme case that was present in the hybrids in either larger or smaller amounts than in either parent (Fig. 3). Myrcene was generally smaller than in either parent. Three monoterpenes (α-terpinene, γ-terpinene and terpinolene) were generally found in hybrids in higher concentrations than either parent (Fig. 3). The hybrids had values of camphene hydrate much like inland parent 267. 4-terpineol was generally intermediate, except for one hybrid with a value of 7.5%. In contrast, α-terpineol is smaller or about the same as its inland 267 parent (Fig. 3). Citronellyl acetate (and

geranyl acetate) is similar to 4-terpineol in that the hybrids are generally intermediate with several outliers that are larger than either parent (Fig. 3). Nearly all the hybrids have only small amounts of germacrene D (and α-cadinol). As in the case of manool (above), this is suggestive of Mendelian inheritance, with absence being dominant.

The yields of oil (on a oven dry wt. basis) are of particular interest to tree breeding in Douglas fir as the amount of oil is a deterrent to deer browsing (Radwan and Ellis 1975). The loss of seedlings to deer browsing is a serious problem in reforestation with Douglas fir (and other conifers) in the Pacific northwest. So it is of interest that coastal fir (226, var. *menziesii*) is very low in oil, whereas inland (267, var. *glauca*) has twice the amount of oil (Table 1). One hybrid had the same concentration as the coastal parent, and several were intermediate (Fig. 4). However, 4 of the 10 hybrids had much larger oil yields and 2 hybrids had 4 times as much oil as their coastal parent (Fig. 4., 0.85%). Clearly, there is sufficient variation in oil yields for considerable selection for resistant Douglas-firs.
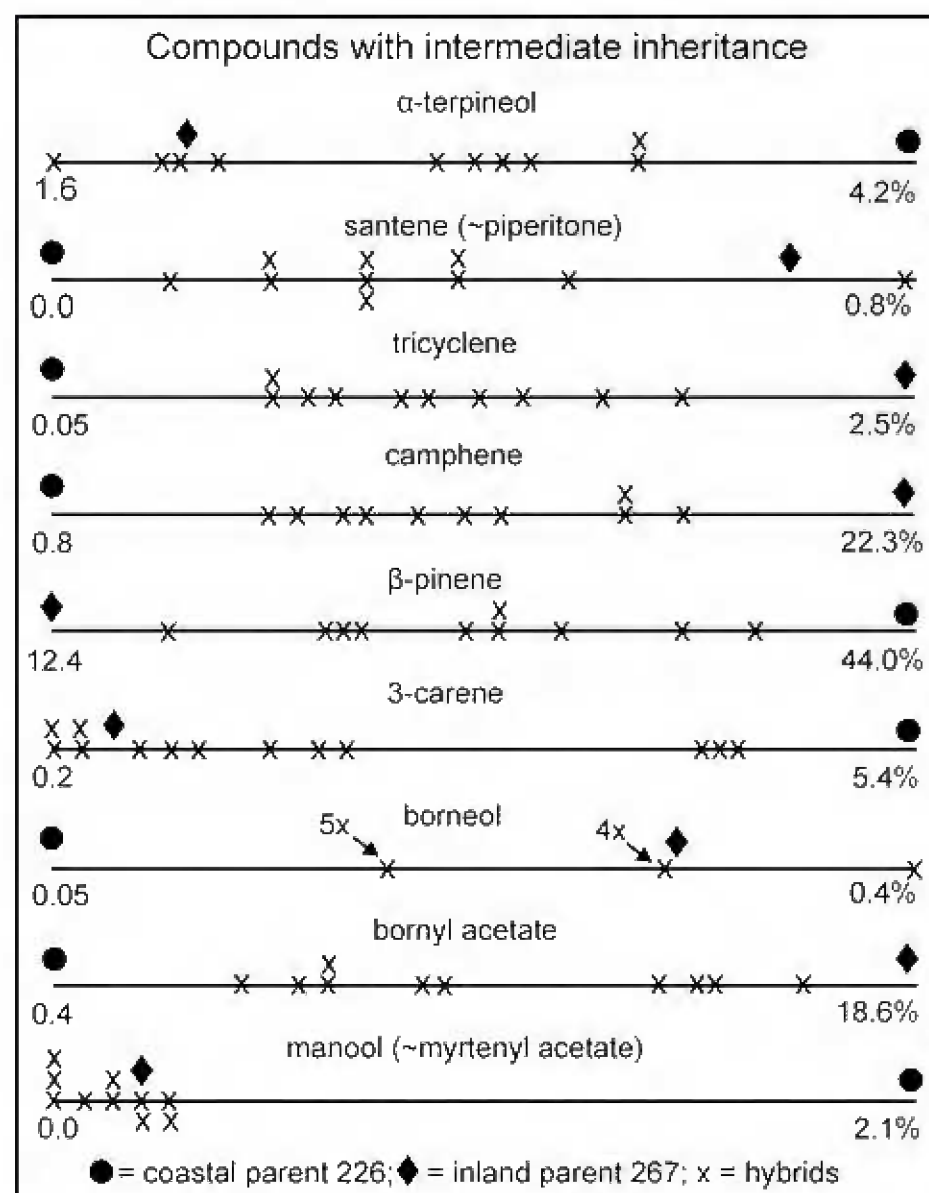

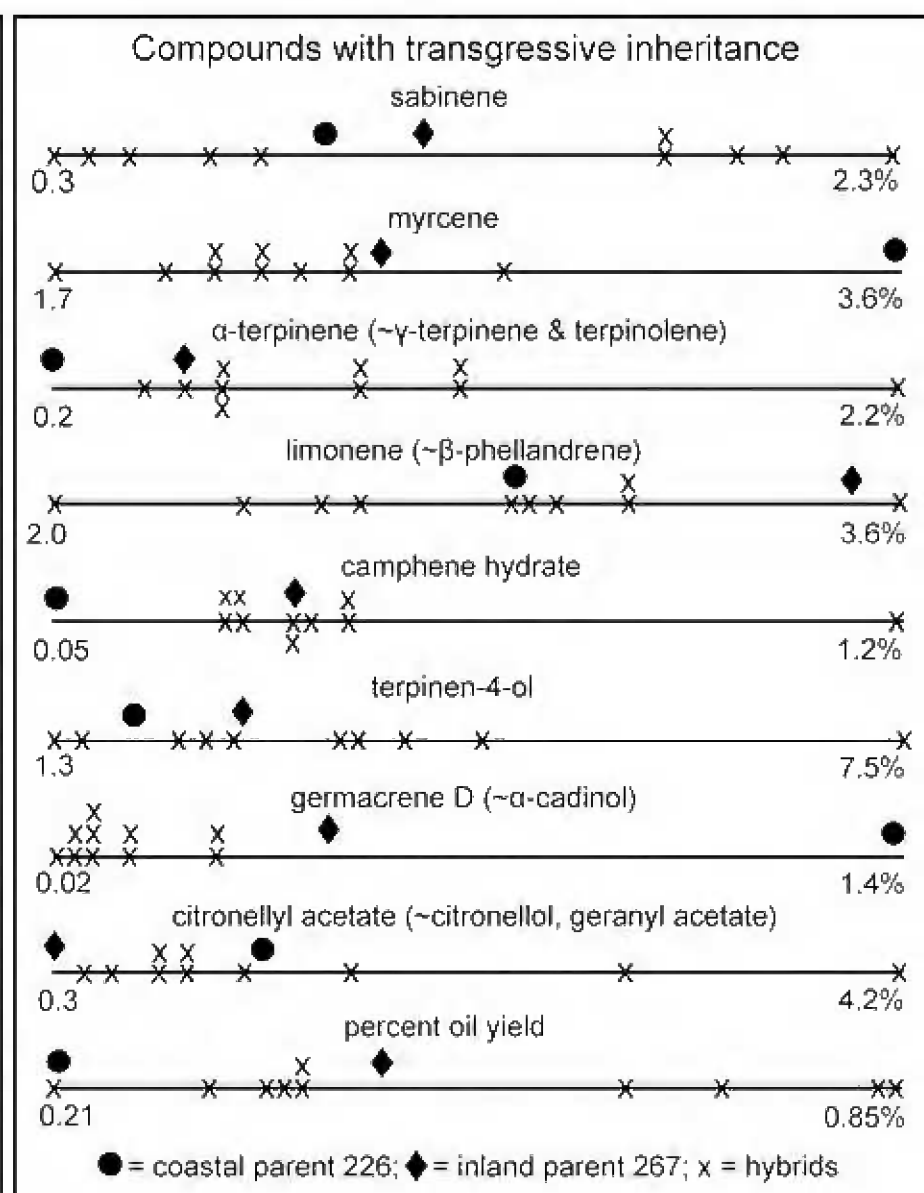Figure 2. Compounds with intermediate inheritance in the hybrids.

Figure 3. Compounds with transgressive inheritance in the hybrids.

PCO ordination of the parents and ten F_1 hybrids (Fig. 4) using character weights of F (from ANOVA) show the parents ordinated on axis 1 (28% of the variance). The hybrids are generally ordinated between the parents but removed on axis 2 (24%). The classical U triangle (see Adams, 1982 for detailed discussion) is not apparent (Fig. 4). Hybrids 1, 3, 5, 7, 8, 10 behave as if the components are quantitatively inherited but hybrids 2, 4, 6, 9 are more like the inland parent 267 (Fig. 4). Based on the computer simulations and morphological data from sunfish hybrids (Adams, 1982), one would conclude that figure 4 indicates a hybrid swarm with back-crossing in one direction (to parent 267). However, this is not correct, as all of the plants (1-10) are F_1 hybrids from a cross between parents 226 and 267.

The problem of transgressive characters overly influencing similarity (and thence PCO ordination) might be corrected by truncating the terpene values to the minimum or maximum value found in either parent. For example, in figure 3, several hybrids had smaller amounts of 4-terpineol than the coastal parent 226 (1.4%) and several hybrids had larger amounts than the inland parent 267 (1.7%). The value of 4-terpineol could be truncated so that the smaller values are not less than 1.4% and the larger values not more than 1.7%. A computer program was written to truncate the data values between the values of the parents. PCO was run using F weighted character matches with truncated data values for the hybrids. The resulting PCO increased the variance removed on PCO 1 axis from 28% to 36%, with no change in the variance on PCO 2 axis (24%). Ordination (Fig. 5) shows the hybrids are more tightly clustered between the parents, but several hybrids still display dominance in inheritance towards the inland parent 267 (Fig. 5). If this were field data, one would likely conclude that both F_1 hybrids (1, 3, 5, 7, 8, 10, Fig. 5) and backcrossed individuals (cf. 2, 4, 6, 9, Fig. 5) are present; which of course, is not the case. This present example in Douglas fir seems to parallel the case in *Cryptomeria japonica* (Fig. 1, above) in which the hybrids displaying dominant inheritance of cedrol/ widdrol/ cis-thujopsene appear as backcrossed individuals in the ordination.
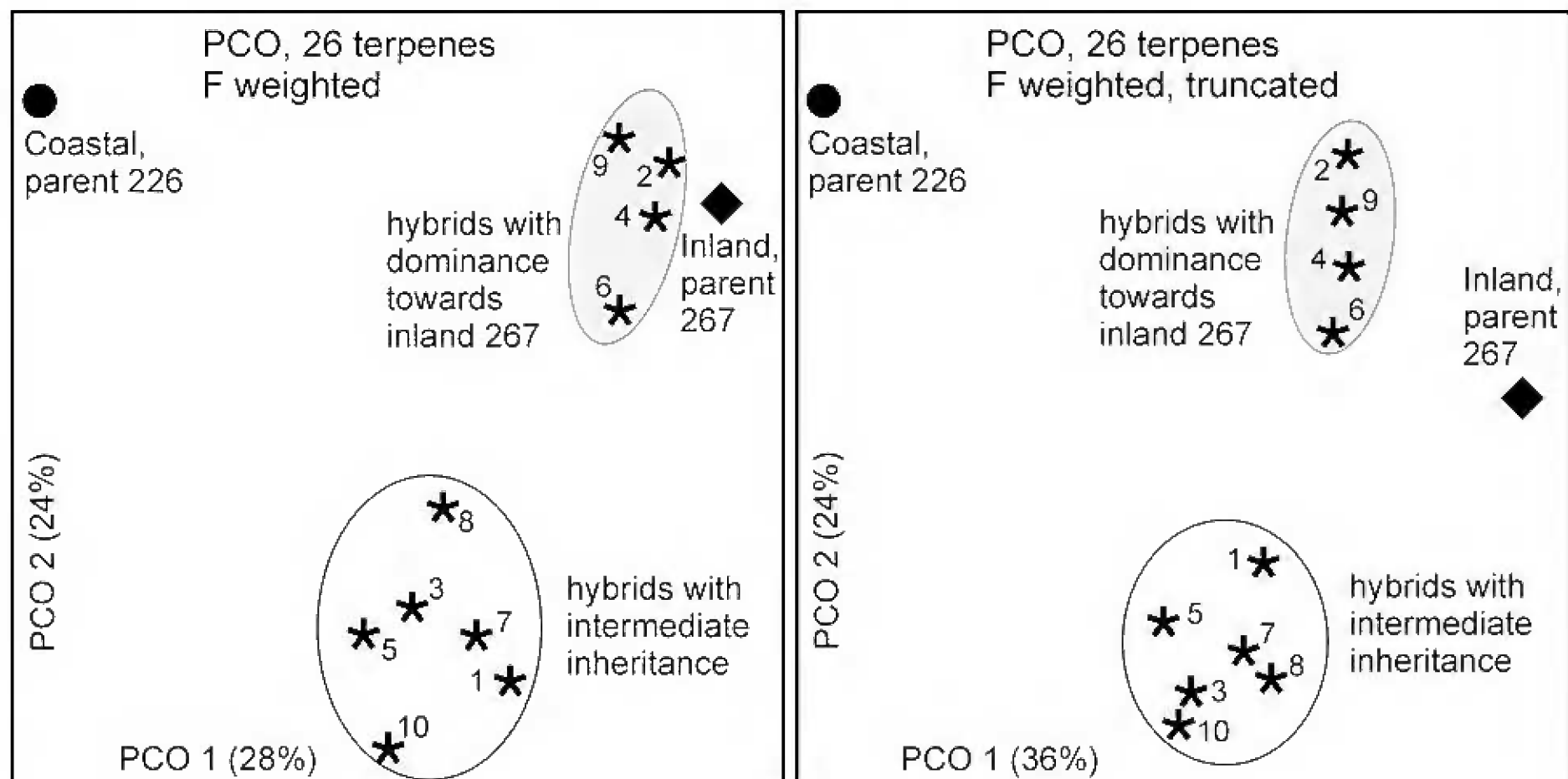

Figure 4. PCO ordination of the parents and hybrids based on 26 terpenes, F weighted.

Figure 5. PCO based on 26 terpenes, F weighted, but truncated between the maximum and minimum values of the parents.

The complementation of the presence / absence of compounds is a very good indication of hybridization. However, if, by chance, most of the components that differentiate two entities have the absence state in one parent, then there may be excessive weight given to this suite of components. Examination of the patterns for the nine intermediate inheritance terpenes (Table 3), reveals the 8 of these had large weights and only α-terpineol had a lower weighting (1.81% total weights). Only two compounds showed clear dominant/ recessive inheritance, but for both of these, the hybrids were like the inland parent 267 (see Fig. 3). So using both manool and myrtenyl acetate would give extra weight to the inland type oil. For the 15 transgressive traits, the hybrids' limonene and β-phellandrene values were similar or transgressive beyond coastal 226 and each terpene had very low weights (0.12, 0.09%). Seven terpenes (Table 3, Fig. 3) displayed transgressive variation, but hybrids' values were more similar to inland parent 267. Using all 7 terpenes probably gave a bias towards inland parent 267. Camphene hydrate was selected to represent this group of 7 terpenes (Table 3). The hybrids in the third group

Table 3. Patterns of variation among coastal parent 226 and inland (interior) parent 267 and hybrids. x denotes the terpene occurrence pattern in coastal parent 226, hybrids and/ or inland parent 267. char wt = F, scaled as % total weight. char wt 1 is the original weighting based on 20 characters (Fs, scaled to % total), char wt 2 is the char weight based on 10 selected characters to balance modes between the parents (Fs, scaled to % total).

cpd	coastal parent 226	hybrids	inland parent 267	char wt 1	char wt 2
intermediate (9)					
α-terpineol	x	x	x	1.81	0
santene	x	x	x	7.27	10.45
piperitone	x	x	x	7.27	10.44
tricyclene	x	x	x	7.00	10.06
camphene	x	x	x	6.75	9.70
β-pinene	x	x	x	3.48	5.00
3-carene	x	x	x	8.99	12.91
borneol	x	x	x	4.55	6.53
bornyl acetate	x	x	x	6.96	10.00
dominant/ recessive (2), 2 hybrids' cpds more like inland parent 267					
manool		x	x	5.90	8.47
myrtenyl acetate		x	x	7.27	0
transgressive (15), 7 hybrids' cpds more like inland parent 267, 2 hybrids' cpds more like coastal 226					
limonene	x-transgressive	x		0.12	0
β-phellandrene	x-transgressive	x		0.09	0
citronellyl acetate	x-transgressive	x	x	4.29	6.17
citronellol	x-transgressive	x	x	7.27	0
geranyl acetate	x-transgressive	x	x	3.85	0
	group total wt. 15.62%				
myrcene		x	x-transgressive	0.56	0
α-terpinene		x	x-transgressive	2.26	0
γ-terpinene		x	x-transgressive	1.10	0
terpinolene		x	x-transgressive	0.05	0
camphene hydrate		x	x-transgressive	5.54	7.96
germacrene D		x	x-transgressive	2.67	0
α-cadinol		x	x-transgressive	2.26	0
	group total wt. 14.44%				
terpinen-4-ol	transgressive past both parents values			0.13	0
% oil yield	transgressive past both parents values			1.60	2.30
sabinene	transgressive past both parents values			0.94	0

(citronellyl acetate, citronellol, geranyl acetate, Table 3) tended to be more like the coastal parent 226. Citronellyl acetate was selected to represent this group. Percent oil yield was selected from the fourth group. Thus, a group of 11 terpenes and percent oil yield was selected for PCO using F wts. and truncated values. The resulting ordination (Fig. 6) shows nearly the same pattern as seen with 26 F weighted, truncated terpenes (Fig. 5). This seems surprising, but examination of the total weighting for the first transgressive group of terpenes like with hybrids like the coastal parent 226 (Table 3) is 15.62% vs. 14.44% for the second group (hybrids like inland parent 267). So selecting citronellyl acetate (wt. = 6.175) and camphene hydrate (wt. = 7.96%) apparently made little difference in the ordination (Fig. 6).

This result is both discouraging and encouraging. Even with the data distributions for both parents and hybrids, selecting a 'better' reduced set of 12 terpenes had little effect. On the other hand, merely running ANOVA between the parents and truncating the transgressive terpenes resulted in a good ordination that is similar to the '12 best terpenes'. In cases involving putative natural hybridization, one may not be able to 'select better terpenes', so the more general approach is promising.

A second set of data was available from crosses between coastal parent, 517 (var. *menziesii*) and an intermediate parent, 521 (possibly a natural hybrid between var. *glauca* and var. *menziesii*). The oils from these parents are shown in table 2 along with some hybrids. Hybrids 1, 5 and 7 are generally intermediate in their composition between the parents (Table 2), whereas the terpenes of hybrids 9 and 10 are more like the coastal parent 517 (C517, Table 2).

Examination of the variation among the hybrids reveals that only 2 terpenes are inherited as intermediate characters (Fig. 7). β-pinene shows the most intermediate pattern of any compound with nearly all values ranging between the parents (Fig. 7). The unknown sesquiterpene alcohol (KI 1627) also presented intermediate values, although several were very similar (near zero) as found in parent 512 (Fig. 7).
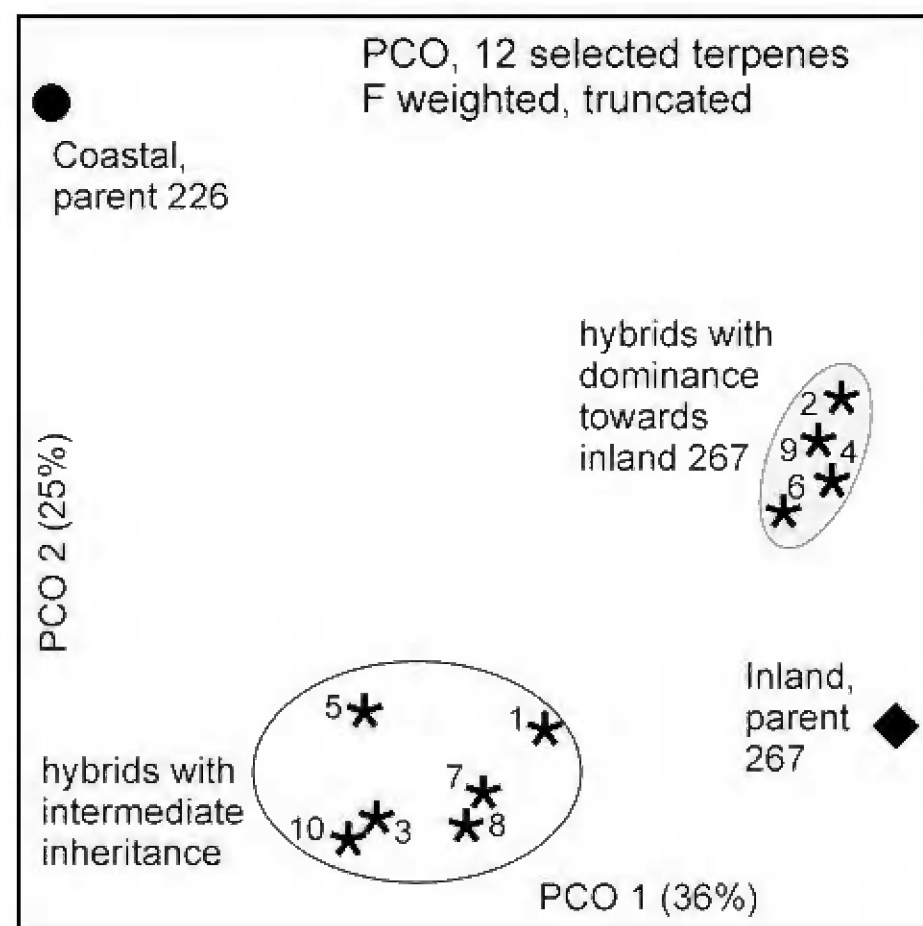

Figure 6. PCO using 12 selected terpenes.

Eight compounds displayed the appearance of dominance/ recessive inheritance: camphene, germacrene D, viridiflorene, bornyl acetate, cembrene, thunbergol, manool and abienol (Fig. 7). Camphene is somewhat intermediate (Fig. 7), with only small amounts like parent 521. Two of the 8 had zero or very small concentrations as found in inland plant 521. Six hybrids had zero or very small amounts as found in coastal parent 517.

Percent oil yield and 9 terpenoids had transgressive variation (Fig. 8). Percent oil yield is interesting as both parents had very low oil yields (0.16, 0.23%, Table 2), yet the hybrids ranged from 0.31 to 1.04% with most yields exceeding either parent (Fig. 8). Again, breeding for increased oil yields for herbivore browsing resistance seems favorable between these parents. Additional research is on-going on in this field of chemo-resistance (Burney and Jacobs, 2012; Kimball et al. 2011). In several cases most of the hybrids either exceeded the parents or were less then either parent (see Fig. 8: α-pinene, 3-carene, terpinolene, 4-terpineol, bornyl acetate, and manool).

PCO using F (from ANOVA) weighted characters removed 37 and 22% percent of the variation among the parents and hybrids on the first two coordinates. Ordination reveals the hybrids are in two groups (Fig. 9), with one group (1, 4, 5, 6, 7, 8) more intermediate between the parents (as expected for hybrids). The second group (2, 3, 9, 10) are still intermediate, but closer to coastal parent 517 (Fig. 9).
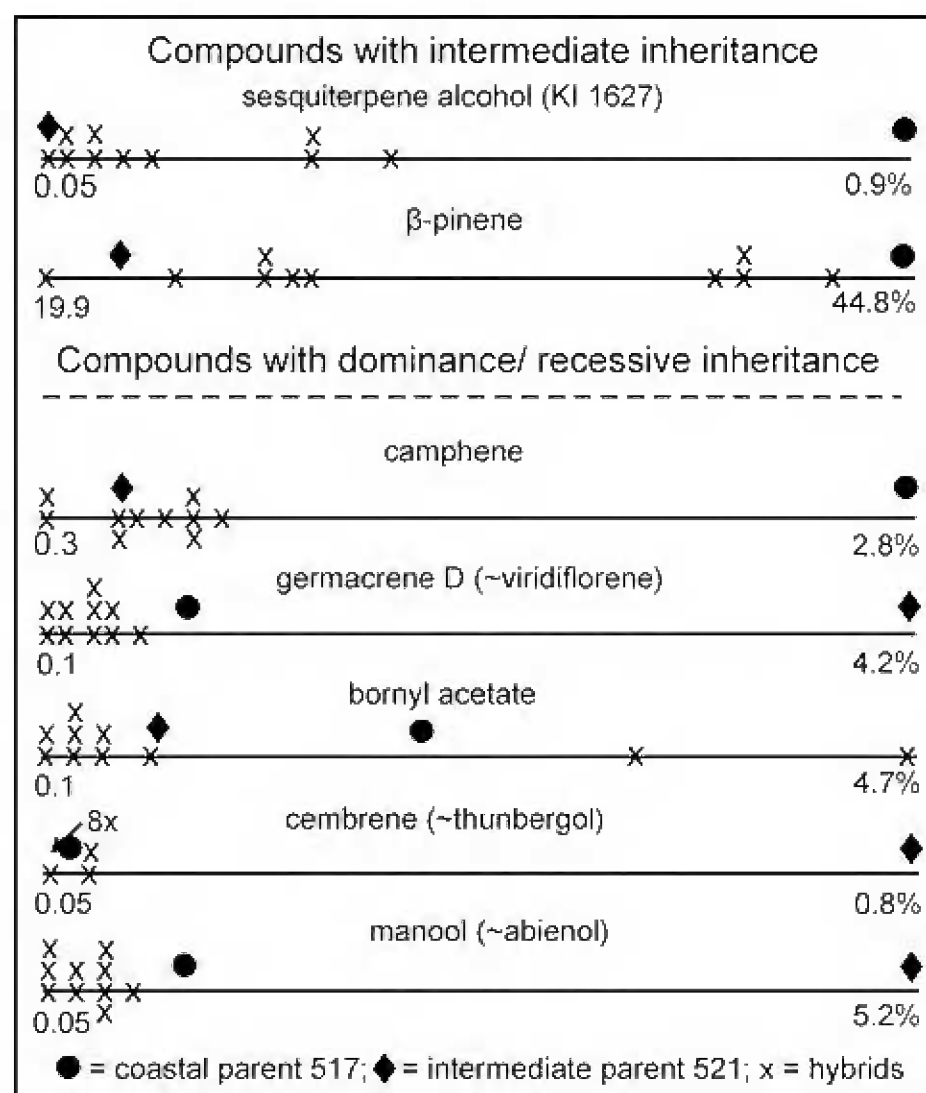
Figure 7. Compounds with intermediate or showing dominance towards one of the parents.
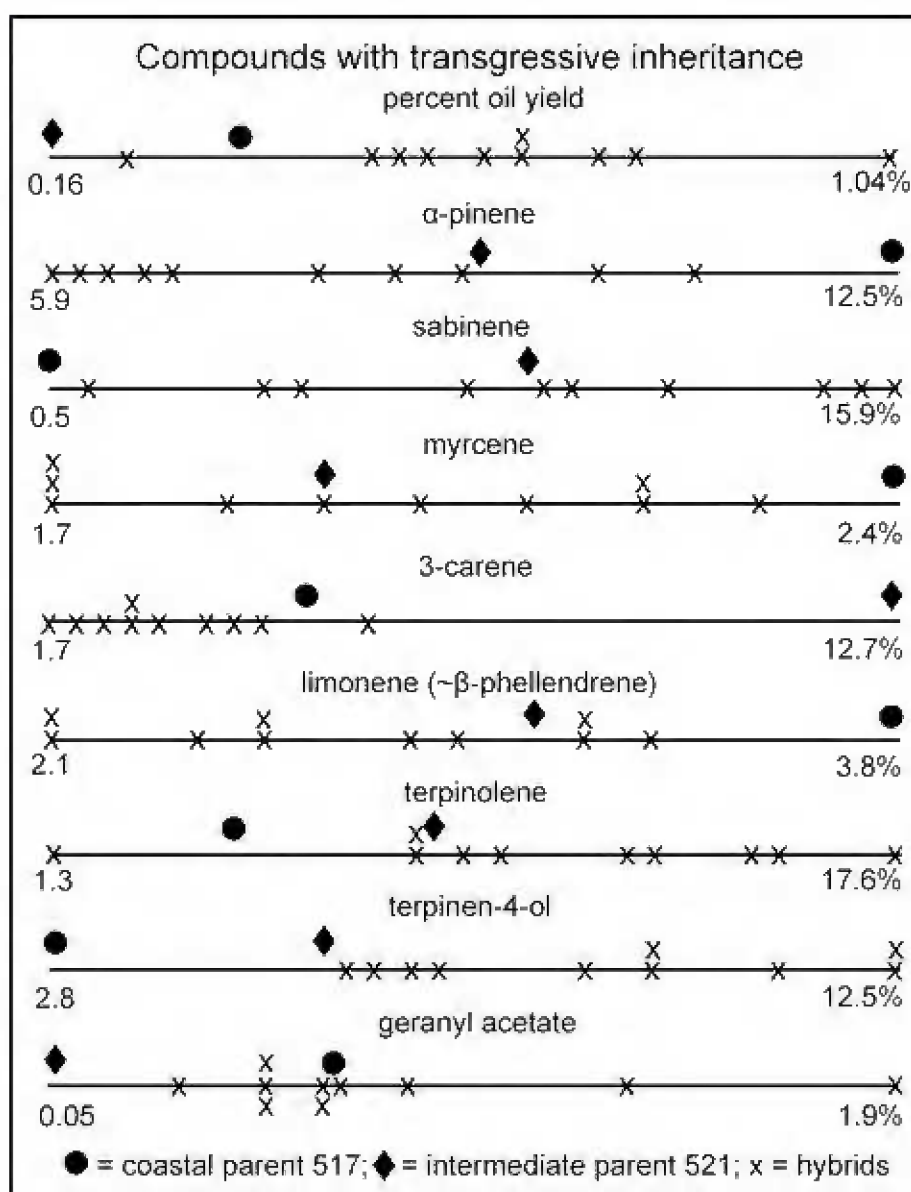
Figure 8. Percent oil yield and 9 terpenoids that had transgressive values in the hybrids.

A second PCO with F weighting and truncated data values removed 46 and 23% of the variance. There is an improvement in the percent variance accounted for in PCO using truncated data, just as with the previous data set (cf. Figs. 4, 5). Ordination shows (Fig. 10) the clusters are a little tighter and the two hybrid groups are now essentially connected into one group. In both the previous cross (coastal 226 x inland 267) and this cross (coastal 517 x intermediate 521), the use of truncated data values appears to improve the detection of hybrids.

Examination of the patterns for the eight dominant/ recessive inheritance terpenes (Table 4), shows only 2 terpenes (camphene, bornyl acetate) represent the pattern of intermediate parent 521 and 6 typify the pattern of coastal parent 517. Thus, if all 8 are used, there is a bias towards terpenes representative of the coastal parent 517. To correct this bias, one could select 2 from each group (camphene, bornyl for parent 521 type), (germacrene D, manool for coastal parent 517 type). Among the transgressive characters, 2 characters were chosen to represent the coastal parent 517 type (percent oil yield, 3-carene) and 2 were chosen to represent the parent 521 type (terpinolene, terpinen-4-ol). Including the 2 terpenes showing intermediate inheritance (β-pinene, KI1627), this gives 10 characters (Table 4) of which 4 are associated with the coastal parent 517 and 4 associated with the parent 521.

PCO using these 10 characters, F weighted, and truncated shows a more intermediate pattern (Fig. 11). Note that the variance removed is very similar to previous ordinations (Figs. 9, 10), yet the placement of the hybrids is more intermediate and in a tighter group. Although this approach seems to be very useful in the present case of known parents and hybrids, it may be difficult to apply to natural field hybridization cases. It is interesting that hybrids 3, 8, 9, 10 are quite separated with F wts (Fig. 9), then

less separated when the transgressive data values are truncated to values of the parents (Fig. 10), and finally using 10 selected terpenes, F weighted, truncated, the hybrids 3, 8, 9, 10 are completely intermingled with hybrids 1, 2, 4, 5, 6, 7 (Fig. 11). Clearly, in this case, selecting representative terpenes from both parents to balance the modes of inheritance benefited the ordination by placing the hybrids in a more intermediate position. This allows the investigator to correctly assign these as hybrids. In addition, this provides for the possibility that in a natural field hybridization case, back-crossed individuals may be ordinated between the hybrids and the back-crossed parent.
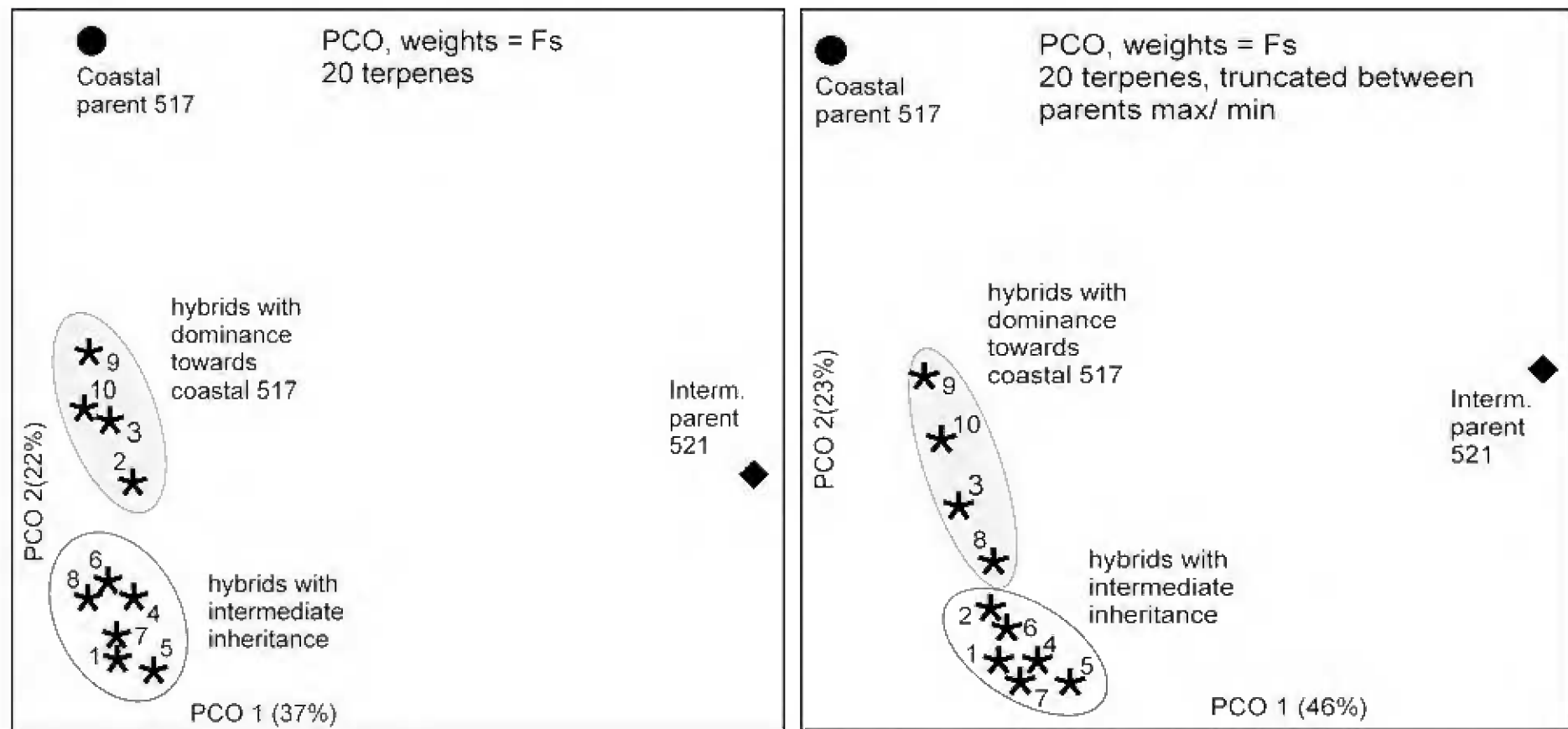

Figure 9. PCO with F weighted, 20 terpenes.

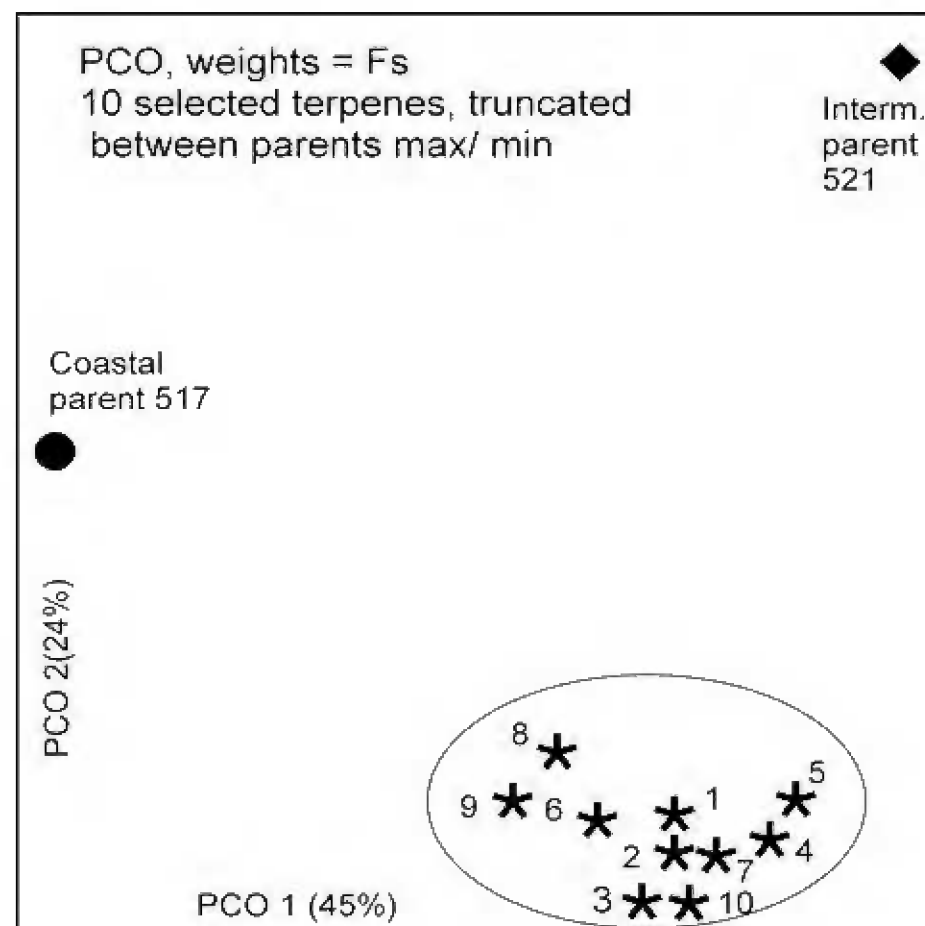Figure 10. PCO with F weighted, 20 terpenes, truncated between parents.

Figure 11. PCO using 10 selected characters to balance representation of modes equally between the parents. Notice the hybrids are in a tighter group and very intermediate between the parents.

Table 4. Patterns of variation among parents 517 and 521 and hybrids. x denotes the terpene occurrence pattern in coastal parent 517, hybrids and/ or parent 521. char wt = F, scaled as % total weight. char wt 1 is the original weighting based on 20 characters (Fs, scaled to % total), char wt 2 is the char weight based on 10 selected characters to balance modes between the parents (Fs, scaled to % total).

cpd	coastal parent 517	hybrids	intermediate parent 521	char wt 1	char wt 2
intermediate (2)					
KI 1627	x	x	x	9.41	20.44
β-pinene	x	x	x	2.44	5.31
dominant/ recessive (8), 2 hybrids like 521, 6 hybrids like 517					
camphene		x	x	7.69	16.70
bornyl acetate		x	x	5.46	11.86
germacrene D	x	x		7.69	16.70
viridiflorene	x	x		8.31	0
cembrene	x	x		9.23	0
thunbergol	x	x		9.93	0
manool	x	x		5.65	12.28
abienol	x	x		6.91	0
transgressive (10), 7 hybrids' cpds more like 521, 3 hybrids' cpds more like 517					
% oil yield	x-transgressive	x		0.65	1.42
3-carene	x-transgressive	x		2.79	6.07
geranyl acetate	x-transgressive	x		8.73	0
			group total wt. 12.17%		
terpinolene		x	x-transgressive	2.02	4.37
terpinen-4-ol		x	x-transgressive	2.23	4.83
α-pinene		x	x-transgressive	0.48	0
sabinene		x	x-transgressive	9.59	0
myrcene		x	x-transgressive	0.28	0
limonene		x	x-transgressive	0.22	0
β-phellandrene		x	x-transgressive	0.27	0
			group total wt. 15.09%		

CONCLUSION

From the previous study of *Cryptomeria japonica* synthetic hybrids (Adams and Tsumura, 2012) and the present study of two crosses of *Pseudotsuga menziesii*, it is clear that the detection of hybridization may prove to be difficult due to the presence of linkage groups (*Cryptomeria japonica*) and/ or the presence of terpenes are inherited as dominant/ recessive traits in which several compounds co-vary in the direction of one of the parents (present study). This study may illustrate inheritance differences between a wide genetic cross (226, var. *menziesii*, x 267, var. *glauca*) and a more narrow cross (517, var. *menziesii*, x 521, intermediate between var. *menziesii* and var. *glauca*, possibly a hybrid). Notice that comparing table 3 (wide cross) and table 4 (narrow cross):

	wide genetic cross	narrow genetic cross
no. intermediate cpds.	9	2
no. dominant/ recessive cpds.	2	8
no. transgressive cpds.	15	10

One might expect more transgressive cpds in a wide genetic cross as there should be more incompatible pathways activated in a wider cross. Thus, the taxonomically wider the cross, the higher the probability that some independently evolved pathways exist in the parental lines. And as a consequence, the hybrids will contain disparate gene regulators, so gene control may be disrupted, leading to over/under expression of some compounds. The presence of more intermediate compounds in the wide cross and more dominant/ recessive compounds in the narrow cross is difficult to explain.

At present, it appears that utilizing F (from ANOVA between the parents) to weight the character matches and truncating transgressive characters back to the range found in the parents, aids in producing ordinations in which the hybrids are intermediate between the parents. The use of selected components that are typical for each parent and using the same numbers of parental representatives helped in ordinating the hybrids intermediate between the parents in one of the two cases examined in this study. In a study of natural field hybridization, the terpenes should be useful in the detection of hybrids but it may be difficult to accurately classify back-crossed or F_2 individuals.

ACKNOWLEDGEMENTS

LITERATURE CITED

Adams, R. P. 1975. Statistical character weighting and similarity stability. Brittonia 27: 305–316.

Adams, R. P. 1982. A comparison of multivariate methods for the detection of hybridization. Taxon 31: 646-661.

Adams, R. P. 1991. Cedarwood oil - Analysis and properties. In: Linskens, H.-F., Jackson, J. F. (eds.). Modern Methods of Plant Analysis, New Series: Oil and Waxes. Springler-Verlag, Berlin. pp. 159–173.

Adams, R. P. 2007. Identification of essential oil components by gas chromatography/ mass spectrometry. fourth ed. Allured Publishing, Carol Stream, IL.

Adams, R. P. and Y. Tsumura. 2012. Multivariate detection of hybridization using conifer terpenes I: Analysis of terpene inheritance patterns in *Cryptomeria japonica* F_1 hybrids Phytologia 94(2): 253-275.

Adams, R. P., J. J. Vargas-Hernández, M. S. González Elizondo, D. Thornburg and F. C. Callahan. Geographic variation in the leaf essential oils of Douglas Fir (*Pseudotsuga menziesii*). 94(2): 199-218.

Burney, O. T., and D. F. Jacobs. 2012. Terpene production and growth of three Pacific Northwest conifers in response to simulated browse and nutrient availability. Trees 26:1331-1342.

Gower, J. C. 1966. Some distance properties of latent root and vector methods used in multivariate analysis. Biometrika 53: 326-338.

Gower, J. C. 1971. A general coefficient of similarity and some of its properties. Biometrics 27: 857-874.

Kimball, B. A., F. Pfund, M. Gourley, D. L. Griffin and J. H. Russell. 2011. Silvicultural attempts to induce browse resitance in conifer seedlings. Int. J. For. Res. Vol. 2011. Article ID 108529, doi:10.1155/2011/108529.

Pohjola, J., R. Hiltunen and M. von Schantz. 2006. Variation and inheritance of terpenes in Scots pine. Flavour Fragrance J. 4: 121-124.

Radwan, M. A. and W. D. Ellis. 1975. Clonal variation in monoterpene hydrocarbons of vapors of Douglas-fir foliage. For. Sci. 21:63-67.

Steele, R. G. D. and J. H. Torrie. 1960. Principles and procedures of Statistics. McGraw-Hill Book Co., NY.

Veldman D. J. 1967. Fortran programming for the behavioral sciences. Holt, Rinehart and Winston Publ., NY.

von Rudloff, E. 1984. Inheritance of leaf oil terpene patterns in selfed progeny and some crosses of coastal Douglas-fir. Silvae Genetica 33: 198-203.

von Rudloff, E. and G. E. Rehfeldt. 1980. Chemosystematic studies in the genus *Pseudotsuga*. IV. Inheritance and geographical variation in the leaf oil terpenes of Douglas-fir from the Pacific Northwest. Can. J. Bot. 58: 546-556.

Table 1. Comparison of leaf oil compositions for *Pseudotsuga menziesii* oils. var. *menziesii*: (coastal parent) C226, var. *glauca* (inland parent) I267, with several hybrids. Compounds in bold face appear to separate the parents, had significant F ratios and were used in numerical analyses.

KI	cpd	C226	I267	F₁ #1	F₁ #3	F₁ #8	F₁ #2	F₁ #9
	% yield	**0.21**	**0.42**	**0.74**	**0.37**	**0.43**	**0.49**	**0.40**
884	**santene**	**-**	**0.7**	**0.3**	**0.2**	**0.3**	**0.8**	**0.5**
921	**tricyclene**	**t**	**2.5**	**1.4**	**0.9**	**1.0**	**1.9**	**1.7**
924	α-thujene	t	t	0.2	0.1	0.2	0.3	0.1
932	α-pinene	16.0	15.1	13.4	14.2	15.1	12.3	13.5
946	**camphene**	**0.8**	**22.3**	**12.0**	**9.0**	**9.7**	**16.9**	**16.3**
969	**sabinene**	**0.7**	**1.2**	**1.8**	**0.8**	**0.5**	**2.3**	**0.3**
974	**β-pinene**	**44.1**	**12.4**	**24.2**	**39.1**	**36.0**	**17.1**	**29.4**
988	**myrcene**	**3.6**	**2.4**	**2.1**	**2.4**	**2.7**	**1.7**	**2.1**
1002	α-phellandrene	-	0.1	0.2	0.1	0.1	0.1	0.1
1008	**δ-3-carene**	**5.4**	**0.4**	**0.5**	**0.7**	**2.0**	**2.1**	**1.2**
1014	**α-terpinene**	**0.2**	**0.5**	**1.2**	**0.6**	**0.6**	**1.0**	**0.4**
1020	p-cymene	0.4	0.3	0.3	0.5	0.5	0.8	0.4
1024	**limonene**	**2.9**	**3.5**	**2.5**	**2.9**	**3.1**	**2.9**	**3.6**
1025	**β-phellandrene**	**3.0**	**3.5**	**2.5**	**2.9**	**3.1**	**2.9**	**3.7**
1032	(Z)-β-ocimene	-	0.3	t	t	t	t	t
1054	**γ-terpinene**	**0.5**	**0.9**	**2.1**	**1.0**	**1.0**	**1.7**	**0.7**
1065	cis-sabinene hydrate	-	t	t	t	t	t	t
1086	**terpinolene**	**2.4**	**2.7**	**5.4**	**3.3**	**3.2**	**5.5**	**2.6**
1089	6-camphenone, isomer	-	t	t	t	t	t	t
1095	linalool	0.2	0.3	0.3	0.3	0.3	t	t
1118	cis-p-menth-2-en-1-ol	t	t	0.2	t	0.1	0.2	t
1118	endo-fenchol	0.2	-	t	0.1	0.2	t	t
1135	trans-pinocarveol	0.3	-	t	-	t	t	t
1136	trans-p-menth-2-en-1-ol	-	t	t	t	t	t	t
1141	camphor	t	t	t	t	t	t	t
1145	**camphene hydrate**	**t**	**0.4**	**0.5**	**0.3**	**0.3**	**0.5**	**0.4**
1165	**borneol**	**t**	**0.3**	**0.2**	**0.2**	**0.3**	**0.3**	**0.3**
1174	**terpinen-4-ol**	**1.4**	**1.7**	**4.3**	**2.6**	**2.2**	**3.7**	**1.3**
1179	p-cymen-8-ol	t	-	t	t	t	t	t
1186	**α-terpineol**	**4.2**	**1.9**	**3.3**	**3.0**	**3.4**	**2.0**	**2.2**
1195	myrtenal	0.4	-	t	t	t	t	t
1196	ethyl octanoate	-	0.1	t	0.3	0.1	t	t
1223	**citronellol**	**0.4**	**-**	**1.4**	**0.8**	**0.6**	**0.7**	**0.4**
1232	thymol, methyl ether	-	0.2	t	-	-	t	t
1249	**piperitone**	**-**	**0.8**	**0.5**	**0.7**	**0.8**	**t**	**t**
1287	**bornyl acetate**	**0.4**	**18.6**	**9.1**	**7.4**	**7.5**	**14.5**	**13.3**
1298	trans-pinocarvyl acetate	0.2	t	t	t	t	t	t
1323	methyl decanoate	-	0.1	t	t	t	t	t
1324	myrtenyl acetate	0.1	-	t	t	t	t	t
1350	**citronellyl acetate**	**1.4**	**0.3**	**3.0**	**0.9**	**0.5**	**1.8**	**0.7**
1357	(2E)-undecenal	0.1	-	t	t	t	t	t
1379	**geranyl acetate**	**0.4**	**0.1**	**1.6**	**0.5**	**0.2**	**0.8**	**0.2**
1395	ethyl decanoate	-	t	t	t	t	t	t
1452	α-humulene	0.2	0.2	t	t	t	t	t
1480	**germacrene D**	**1.4**	**0.5**	**t**	**t**	**t**	**0.2**	**0.2**
1522	δ-cadinene	0.2	0.1	t	t	t	t	t
1608	humulene epoxide II	0.1	t	t	t	t	t	t
1616	43,81,161,222	0.7	0.5	0.5	0.5	0.5	0.6	0.5

1627	43,119,159,220	0.8	0.8	-	0.4	0.4	0.7	0.6
1638	epi-α-cadinol	0.2	0.4	-	t	t	0.1	t
1638	epi-α-muurolol	0.2	0.4	-	t	t	0.1	t
1644	α-muurolol	t	t	-	t	t	t	t
1652	**α-cadinol**	**1.0**	**0.4**	**t**	**0.2**	**0.1**	**0.4**	**0.4**
1814	hexadecanal	t	0.1	0.6	0.7	0.5	0.3	t
1937	cembrene	0.1	0.2	t	t	t	t	t
1943	iso-cembrene	t	0.1	t	t	t	t	t
1987	manoyl oxide	0.2	t	t	t	t	t	t
2048	thunbergol	0.1	0.2	t	t	t	t	t
2056	**manool**	**2.1**	**0.2**	**t**	**0.2**	**0.3**	**t**	**0.1**
2300	tricosane(C23)	0.5	0.2	0.2	0.2	0.1	0.1	0.3

KI = Kovat's Retention Index on DB-5(=SE54) column using alkanes. Compositional values less than 0.1% are denoted as traces (t). Unidentified components less than 0.5% are not reported.

Table 2. Comparison of leaf oil compositions for *Pseudotsuga menziesii* oils. var. *menziesii*: coastal parent - C517, intermediate - I521, and several hybrids. Compounds in bold face appear to separate the parents and were used in numerical analyses.

KI	cpd	C517	I521	F₁ #1	F₁ #5	F₁ #7	F₁ #9	F₁ #10
	% yield	**0.23**	**0.16**	**1.04**	**0.64**	**0.73**	**0.31**	**0.49**
884	santene	t	t	t	-	-	t	t
921	tricyclene	0.3	t	t	t	t	t	t
924	α-thujene	0.1	0.2	0.5	0.7	0.5	0.3	0.4
932	**α-pinene**	**12.5**	**9.2**	**6.9**	**6.1**	**6.7**	**8.9**	**8.5**
946	**camphene**	**2.8**	**0.4**	**0.4**	**0.3**	**0.4**	**0.6**	**0.6**
969	**sabinene**	**0.5**	**9.8**	**11.8**	**15.2**	**9.6**	**3.5**	**1.2**
974	**β-pinene**	**44.8**	**21.1**	**26.7**	**19.9**	**25.3**	**39.2**	**43.0**
988	**myrcene**	**2.4**	**1.9**	**1.7**	**1.7**	**1.8**	**2.0**	**2.1**
1002	α-phellandrene	0.1	0.2	0.2	0.4	0.4	0.2	0.3
1008	**δ-3-carene**	**5.6**	**12.7**	**9.5**	**2.5**	**1.7**	**3.6**	**3.5**
1014	α-terpinene	0.8	0.7	2.5	4.1	3.5	1.6	1.9
1020	p-cymene	0.2	0.4	0.5	0.9	0.9	1.3	0.9
1024	**limonene**	**3.8**	**3.1**	**2.1**	**2.3**	**2.5**	**2.9**	**2.8**
1025	**β-phellandrene**	**3.9**	**3.1**	**2.1**	**2.3**	**2.5**	**2.9**	**2.8**
1032	(Z)-β-ocimene	t	t	0.1	t	t	t	t
1044	(E)-β-ocimene	t	0.3	0.1	t	0.4	t	0.6
1054	γ-terpinene	1.3	1.5	4.2	6.5	5.4	2.7	3.2
1065	cis-sabinene hydrate	t	t	0.4	0.3	0.2	0.2	0.2
1086	**terpinolene**	**4.3**	**8.4**	**11.2**	**17.6**	**13.6**	**8.0**	**10.2**
1089	6-camphenone, isomer	t	t	t	t	t	t	t
1095	linalool	0.2	0.5	0.5	0.3	0.3	0.3	0.3
1118	cis-p-menth-2-en-1-ol	0.1	t	0.6	0.6	0.6	0.4	0.4
1118	endo-fenchol	t	t	t	t	t	0.2	t
1135	trans-pinocarveol	t	t	0.4	0.5	0.4	0.3	0.3
1136	trans-p-menth-2-en-1-ol	t	t	t	t	t	t	t
1141	camphor	t	t	t	t	t	t	t
1145	camphene hydrate	0.2	t	0.2	t	0.1	0.2	0.1
1165	borneol	t	t	t	t	t	t	t
1172	cis-pinocamphone	t	0.4	t	t	t	t	t
1174	**terpinen-4-ol**	**2.8**	**5.7**	**9.4**	**12.4**	**12.4**	**6.7**	**7.1**
1179	p-cymen-8-ol	t	0.2	t	0.2	0.2	0.2	0.2
1186	α-terpineol	4.4	3.5	2.3	2.0	3.3	3.6	2.7
1195	myrtenal	t	t	0.2	t	t	t	t
1196	ethyl octanoate	t	t	0.2	0.2	0.2	0.2	0.1
1223	citronellol	t	t	2.1	0.2	1.1	0.3	0.8
1232	thymol, methyl ether	t	t	t	t	t	t	t
1249	piperitone	t	t	0.1	t	t	0.2	t
1287	**bornyl acetate**	**2.3**	**0.6**	**0.1**	**0.1**	**0.2**	**4.7**	**0.3**
1298	trans-pinocarvyl acetate	t	t	t	t	t	t	t
1323	methyl decanoate	t	t	t	t	t	t	t
1324	myrtenyl acetate	t	t	t	t	t	t	t
1350	citronellyl acetate	0.5	0.3	3.1	0.4	1.3	0.7	1.1
1357	(2E)-undecenal	t	t	t	t	t	t	t
1379	**geranyl acetate**	**0.6**	**t**	**1.9**	**0.3**	**1.3**	**0.5**	**0.8**
1395	ethyl decanoate	t	t	t	t	t	t	t
1452	α-humulene	0.1	t	t	t	t	t	t
1480	**germacrene D**	**0.6**	**4.2**	**0.1**	**0.2**	**0.1**	**0.1**	**0.2**
1496	**viridiflorene**	**t**	**0.5**	**0.1**	**t**	**0.1**	**0.1**	**t**

1508	germacrene A	t	0.4	t	t	t	t	t
1522	δ-cadinene	0.2	t	t	t	t	0.1	t
1608	humulene epoxide II	t	t	t	t	t	t	t
1616	<u>43</u>,81,161,222	t	-	t	t	t	t	t
1627	**<u>43</u>,119,159,220**	**0.9**	**-**	**0.1**	**t**	**0.1**	**0.3**	**0.1**
1638	epi-α-cadinol	0.1	t	t	t	0.1	t	t
1638	epi-α-muurolol	0.2	t	t	t	t	t	t
1644	α-muurolol	t	t	t	t	t	t	t
1652	α-cadinol	0.4	0.3	0.1	t	t	0.3	0.4
1814	hexadecanal	t	t	t	t	t	0.1	t
1937	**cembrene**	**t**	**0.8**	**t**	**t**	**t**	**t**	**t**
1943	iso-cembrene	t	0.3	t	t	t	t	t
1987	manoyl oxide	0.3	0.3	t	t	t	t	t
2048	**thunbergol**	**t**	**1.5**	**t**	**t**	**t**	**t**	**t**
2056	**manool**	**1.3**	**5.2**	**0.4**	**0.1**	**0.3**	**0.2**	**0.3**
2149	**abienol**	**0.2**	**1.1**	**t**	**t**	**t**	**t**	**t**
2300	tricosane(C23)	0.1	0.3	t	t	t	t	t

KI = Kovat's Retention Index on DB-5(=SE54) column using alkanes. Compositional values less than 0.1% are denoted as traces (t). Unidentified components less than 0.5% are not reported.